Cryo-electron tomography of mouse centrosomes

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Centrosomes are the major microtubules organizing centers of animal cells and their structure is remarkably conserved throughout evolution. They are composed of two centrioles surrounded by a mesh of proteins called the pericentriolar material. Centrioles consist of a cylinder surrounded by nine sets of microtubules triplets. Centriole duplication is tightly regulated during the cell cycle. In KE37 human cells, it has been recently shown that mother and daughter centrioles are transiently linked by fibers (connecting stalk) at the initiation of duplication [1]. Presently, we are interested to identify the molecular players contributing to the formation of the connecting stalks. To this purpose we are using mice strains that over-express centriole-duplication proteins to further characterize, by cryo-electron-tomography, the initial steps of centriolar duplication in biological conditions closer to the native state.

As a proof of concept and with the aim of characterizing mouse centrioles, we have purified centrosomes from the TTA1.6 lymphocyte cell line. Cryo-electron tomograms of mouse centrosomes show that they contain two centrioles with the canonical structure: centriole appendages and nine microtubule triplets where the cap on the A-microtubule can be identified depending on the maturation stage. This validates the use of mice centrosomes to study centriole duplication by cryo-electron tomograms.

References:

[1] Guichard et al, EMBO J. 2010 May 5;29(9):1565-72.

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